

Ability of an orally administered lactobacilli preparation to improve the quality of the neovaginal microflora in male to female transsexual women

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ABSTRACT

Objective: Lactobacilli have been found in the neovagina of very low numbers of transsexual women. We undertook this study to determine whether an orally administered preparation of four lactobacilli strains exerts some measurable effect on the neovaginal microflora of female transsexuals.

Study design: 60 male to female transsexual women with penile linked neovagina were randomised into two groups. Women in the intervention group ($n = 33$) received oral probiotic capsules and women in the control group ($n = 27$) placebo in for 7 days. Swabs of the neovagina were taken before and after the therapy.

Results: Comparing the first and second swabs, we observed a significant improvement of the Nugent score in the intervention group 16 (48.5%) vs. low improvement in control group 4 (14.8%) ($p < 0.006$). The neovaginal microflora was significantly enriched with lactobacilli after oral supplementation compared to placebo. In the intervention group, an increase by $10,000 \pm 600$ colony forming units (CFU) of presumptive lactobacilli was observed, compared with an increase by 1600 ± 100 CFU in the control group ($p < 0.0001$). When measured by real-time PCR (c/ml), lactobacilli increased by 1400 ± 100 c/ml in the intervention group and 300 ± 100 c/ml in the control group ($p < 0.0001$).

Conclusion: There was an improvement of vaginal lactobacilli microflora after of oral supplementation with lactobacilli strains in transsexual women.

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1. Introduction

The microflora of male to female transsexual women is a complex symbiosis of aerobic and anaerobic species with a very limited number of lactobacilli. It has substantial similarity to the abnormal vaginal microflora characteristic of bacterial vaginosis (BV) [1,2]. Weyers et al. reported that, although transsexual women show serum oestradiol levels comparable to those of postmenopausal women taking oestrogen replacement therapy, their neovaginal environment does not support the growth of lactobacilli [1]. In one study [1], only one of thirty transsexual women had neovaginal colonisation with lactobacilli. Another study of transsexual women, the same authors [2] found a neovaginal lactobacilli colonisation rate of 4%.

Several bacterial species are able to colonise both the gastrointestinal and reproductive tracts. The rectum may play an important role as a source of organisms that colonise the vagina [3]. The gut has been suggested as a reservoir for vaginal colonisation by *Lactobacillus* spp., thereby contributing to the maintenance of a normal vaginal microbiota [4,5]. A recent study has shown that 80% of pregnant women and 40% of postmenopausal women had the same *Lactobacillus* species in both the vagina and rectum [6]. Due to their anatomical and hormonal similarity, a transsexual men-to-women population receiving hormone substitution therapy may be assumed to be comparable with a general female population for investigations of vaginal lactobacilli colonisation.

Some pathogenic bacteria infecting the female urogenital tract emerge from the woman's own intestinal microbiota ascending along the perineum to the vagina. Local substitution with lactobacilli has been shown to significantly enhance the restoration of the vaginal microflora, leading to a significant reduction in the recurrence of urinary tract infections [7,8]. Reid et al. used species-specific PCR-amplification to demonstrate

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that *L. rhamnosus* and *L. fermentum* strains can be delivered to the vagina when administered orally, and found a significant increase in vaginal lactobacilli in treated subjects versus controls [9]. Morelli et al. also showed, using genetic identification techniques, that *L. rhamnosus* and *L. fermentum* strains can be delivered to the vaginal environment even when taken orally [10]. Both studies were performed in healthy premenopausal women. Oral administration of a lactobacilli preparation is a new concept for the restitution of a normal vaginal microflora [11]. We assume that the rectum could play the important role in formation of neovaginal microflora in transsexual women and that selected intestinal lactobacilli also colonise the neovagina, ascending along the perineum. We undertook this prospective, randomised, double-blind, placebo-controlled study to determine whether an orally administered preparation of four lactobacilli strains exerts a measurable effect on the neovaginal microflora of transsexual women.

2. Materials and methods

This prospective, randomised, placebo-controlled, double-blind study was performed with the approval of the Ethics Committee of the Medical University of Vienna (EK 982/2010) in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice. Informed consent was obtained from all study participants prior to enrolment.

We recruited male-to-female transsexual women on an ongoing basis from among transsexual outpatients of the Medical University of Vienna. We included all participants that had undergone sex reassignment surgery (SRS) with the inverted penile skin flap technique [12] at least 1 year before enrolment. All transsexual women were being treated according to the Standards of Care of the World Professional Association of Transgender Health (WPATH) [13]. Exclusion criteria for this study were clinical signs of vaginal or urinary tract infection, abnormal neovaginal discharge, neoplasia, bleeding, diarrhoea, constipation, rectal pathologies including haemorrhoids, and antibiotic therapy in the 4 weeks before enrolment.

At baseline, an initial neovaginal smear was obtained from every male-to-female transsexual woman, after she gave written informed consent, and transferred to a microscopy slide. Smears were Gram-stained and evaluated using the entire 10-grade scale of the Nugent scoring system [14] at an experienced central laboratory. All participants were randomised using a computer-generated randomisation list. Transsexual women in the intervention group received oral probiotic capsules twice daily for 7 days, with each capsule containing four lyophilised *Lactobacillus* strains belonging to the species *L. crispatus*, *L. rhamnosus*, *L. jensenii* and *L. gasseri* (Table 1). Transsexual women in the control group received an identical-looking oral potato-maltodextrin-based placebo twice daily for 7 days. The second and third neovaginal swabs for Nugent scoring and culture were taken on the day after administration of the last capsule and two weeks later.

Table 1
Study medication.

Composition	Guaranteed viable cell count (CFU/dose)
<i>L. crispatus</i> LbV88 (DSM 22566)	1×10^9
<i>L. rhamnosus</i> LbV96 (DSM 22560)	1×10^9
<i>L. jensenii</i> LbV116 (DSM 22567)	0.2×10^9
<i>L. gasseri</i> LbV150N (DSM 22583)	0.3×10^9
Potato maltodextrin	
Insoluble dietary fibre	
Silicium dioxide	

For the cultural and molecular determination of lactobacilli, the swabs were agitated in 1 ml sterile phosphate buffered saline (PBS) for 1 min. An aliquot of 100 µl was diluted 10-fold in PBS, and viable cell counts of presumptive lactobacilli were determined by plating on MRS agar supplemented with 0.5 g/l cysteine hydrochloride and incubating at 37 °C at anaerobic conditions (80% N₂, 10% CO₂, 10% H₂) for 48 h.

The remaining PBS buffer suspensions of the swabs were frozen at -80 °C and then subjected to DNA extraction and real-time PCR-based quantification applying the Sacace™ Bacterial Vaginosis Real-TM Quant kit (Sacace Biotechnologies Srl, Como, Italy) for RotorGene™ 3000/6000/Q (Corbett Research, Mortlake, Australia, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The kit includes the DNA extraction procedure from the specimen and the real-time PCR detection based on fluorescent-dye-marked reporter probes specific for the DNA of all bacteria (Cy5/red), lactobacilli (ROX/orange), *Gardnerella vaginalis* (FAM/green) and *Atopobium vaginae* (JOE/yellow). The results for presumptive lactobacilli are shown in copies/ml (c/ml).

The primary efficacy variable was the change in the Nugent score between baseline and the end of treatment (improvement or no improvement in second swab). The secondary efficacy variable was the evaluation of the lactobacilli concentration by both culture and real-time PCR before and after treatment.

2.1. Statistical analysis

The proportion of subjects with score changes between baseline and the end of the study was compared between treatment groups using a chi-square test. A p-value <0.05 (two-sided) was considered statistically significant. Mean Nugent scores were compared descriptively using the Mann-Whitney U-test.

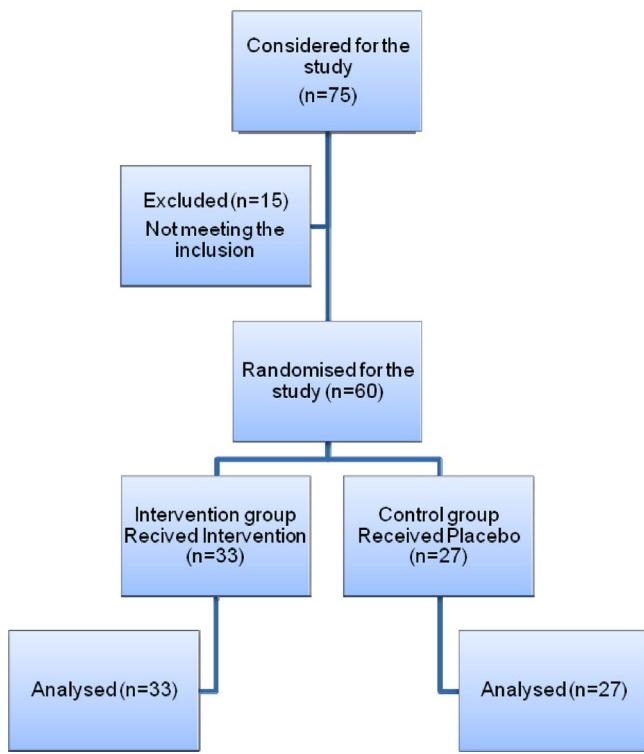
Because this was a pilot study and there was no historical estimate of the proportion of participants showing a clinical benefit from treatment with lactobacilli, the sample size calculation was based on an estimated percentage on the primary efficacy endpoint, i.e. a Nugent score improvement. Considering the potential clinical relevance and assuming an improvement in the Nugent score in more than 50% of women in the intervention group and a possible improvement in a maximum of 5 women in the control group (16.7%), a sample size of 30 subjects per group was considered sufficient to show a statistically significant superiority with a power of at least 80%. SPSS version 11.0 was used for statistical analyses.

3. Results

Between December 2011 and March 2012, a total of 75 male-to-female transsexual women of Caucasian origin were screened for inclusion into the study. The mean age of the women was 41.2 ± 13 years, mean height was 176.5 ± 6.4 cm, mean weight was 82.3 ± 19.9 kg; and the mean time since SRS was 5.2 ± 4.8 years. The flow of participants through the study is illustrated in Fig. 1.

Sixty transsexual women fulfilling all eligibility criteria were included in the final analyses, 33 in the intervention group and 27 in the control group. Based on the first neovaginal smear, a Nugent score of 0–3 was identified in 10 women (30%) in the intervention group and in 8 women (30%) in the control group. An abnormal neovaginal bacterial microflora with a Nugent score of 4–10 in the first smear was detected in 23 women (70%) in the intervention group and in 19 women (70%) in the control group, without a statistical difference between the groups ($p < 0.371$).

Analysis of the first swab showed a median Nugent score of 5 in both groups. Analysis of the second swab after one week of oral lactobacilli administration showed a median Nugent score of 5 in the intervention group and 6 in the control group. Another two

**Fig. 1.** Flow of participants through the study.**Table 2**

Improvement of Nugent score between swabs 1 and 2 and between swabs 2 and 3 in the intervention and control groups.

	Study medication		Placebo		Total	
	N	%	N	%	N	%
Swab 1 vs. 2^a						
Significant improvement	16	48.5	4	14.8	20	33.3
No significant improvement	17	51.5	22	81.5	39	65
Not evaluable	0	0	1	3.7	1	1.7
Total	33	55.0	27	45.0	60	100.0
Swab 2 vs. 3^b						
Significant improvement	7	21.2	9	33.3	16	26.7
No significant improvement	25	75.8	15	55.6	40	66.7
Not evaluable	1	3	3	11.1	4	6.7
Total	28	53.8	24	46.2	52	100.0

^a Chi Square Test: $p = 0.006$.^b Chi Square Test: $p = 0.204$.

weeks later, the median Nugent score was 6 in both groups. Comparison of differences in Nugent score between the first and second swabs showed a significant difference ($p < 0.02$) of -0.18 in the intervention group and $+0.92$ in the control group.

A significant improvement in the Nugent score was seen in 16 women (48.5%) in the intervention group and in 4 women (14.8%) in the control group ($p < 0.006$; **Table 2**).

The differences in presumptive lactobacilli concentrations between the first and second swabs were measured by culture in both groups. In the intervention group, an increase by $10,000 \pm 600$ colony forming units (CFU) of presumptive lactobacilli was seen, compared with an increase by 1600 ± 100 CFU in the control group ($p < 0.0001$). When measured by real-time PCR (c/ml), lactobacilli increases of 1400 ± 100 c/ml in the intervention group

and 300 ± 100 c/ml in the control group ($p < 0.0001$) were seen between the first and second swabs.

When all transsexual women with a normal neovaginal microflora Nugent score of 0–3 at baseline were excluded ($n = 18$), an improvement in the Nugent score was seen in the intervention group ($n = 23$), but not in the control group ($n = 19$). When looking only at women with baseline BV (i.e., a Nugent score > 7 ; $n = 22$), a change to an intermediate microflora was seen in the intervention group but not in the control group.

4. Comment

The results of this prospective randomised controlled study show that oral administration of *L. crispatus*, *L. rhamnosus*, *L. jensenii* and *L. gasseri* significantly improved the neovaginal microflora and reduced the Nugent score in a group of transsexual women. Also, the microflora was significantly enriched with lactobacilli after oral supplementation compared to placebo. The combination of *Lactobacillus* spp. used in this study is the only one published as the physiologic mixture of female vaginal lactobacilli microflora [15]. We used an innovative probiotic lactobacilli composition containing four of the most common lactobacilli isolated from the microflora of healthy women's vaginas [15] for treatment of 7 days' duration. Weyers et al. reported that colonisation of the neovagina of transsexual women with lactobacilli is minimal [1,2]. According to Nugent, an intermediate vaginal microflora is defined by a reduction and BV by an absence of lactobacilli with the presence of Gram negative bacteria in both cases [14]. The small number of publications on the standard neovaginal microflora and the near lack of evidence of lactic acid bacteria in the transsexual genital tract area are a challenge for investigations in this population. While transsexual women have normal female anatomy, there is no uterus and no connection of the neovagina to the pelvic cavity, which is why the risk of pelvic inflammatory disease is low. We were therefore able to include all transsexual women without clinical signs of infection, including those with asymptomatic BV. To our knowledge, this is the first study to allow a direct assessment of the comparative effect of oral probiotic lactobacilli and placebo on BV.

The gastrointestinal tract plays an important role as a reservoir for the vaginal colonisation by *Lactobacillus* spp. [4–6]. Both vaginal and oral applications of lactobacilli have been shown to improve the vaginal microflora of both pre- and post-menopausal women [3,9–11]. The results of this study indicate that oral lactobacilli have a similar effect on the neovaginal flora of transsexual women. Descriptive analyses of the difference in Nugent score showed a reduction of -0.18 in the intervention group and an increase of $+0.92$ in the control group.

We found a significant improvement in the Nugent score in 48.5% of women in the intervention group, compared with only 14.8% in the control group. Lactobacilli concentrations assessed by culture and real-time PCR were 5–6 times higher in the intervention than in the control group, with these differences being statistically significant.

The sample size calculation in this study was based on neovaginal lactobacilli colonization rates of up to 4% reported in the literature [1,2]. In the present study, however, 30% of the women in both the intervention and control groups had a normal lactobacillus microflora (Nugent score ≤ 3). This was an unexpected finding contrasting with the current literature [1,2]. Because oral lactobacillus supplementation cannot be expected to change a neovaginal microflora dominated by lactobacilli, this unexpectedly high proportion of women with a normal lactobacillus flora may have led to an underestimation of the treatment effect. We therefore carried out a subgroup analysis including only women with a baseline Nugent score above 4, corresponding to either an

intermediate microflora or BV. Even then, after 7 days of treatment with oral lactobacilli, we found an improvement in the Nugent score in the intervention group and no change in the control group. The results of this subgroup analysis are comparable with the results of one of our earlier studies on the effect of lactobacilli on postmenopausal women, which showed an improvement in Nugent scores [11]. In contrast to the previous study with a lactobacilli treatment duration of 14 days, however, the improvement in the current was already seen after 7 days of oral lactobacilli. The renewed increase of the Nugent score two weeks after the end of oral therapy indicates that extended oral probiotic therapy may be necessary to maintain a lactobacilli-dominated microbiota.

This study had several limitations. With a specific study group of male to female transsexual women and very limited number of patients visiting our clinic we could observe only a small sample size in our study. The therapy duration was limited to 7 days: we assume that longer treatment with probiotics could obtain a better outcome. Microbiology analyses of CFU's and c/ml were presented only for presumptive lactobacilli. In the next step we will include other bacteria with similar colony characteristics, such as Gardnerella vaginalis and Atopobium vaginæ to present more detailed data. This study is first to observe male to female transsexual women using probiotics and we are aware of our initial oversights.

In summary, this first study on the effect of oral probiotics on the neovaginal microflora of transsexual women found that oral administration of lactobacilli resulted in a significant improvement in the Nugent score and a change of the neovaginal microflora. These observations are consistent with previous results obtained in pre- and post-menopausal women. The increase of the Nugent score two weeks after the end of oral therapy provides a possible need for extended oral probiotic therapy for maintenance of a lactobacilli-dominated microbiota. In addition, this study shows that even asymptomatic BV may be improved to a normal microflora by 7 days of oral supplementation of lactobacilli.

Authors contributions

Ulrike Kaufmann: First author, organisation and preparation of the research clinical support and patient service, writing the paper; Konrad J. Domig: Microbiology research and writing the paper; Christina I. Lippitsch: Microbiology research; Manuel Kraler: Microbiology research; Julian Marschalek: Clinical support;

Wolfgang Kneifel: Microbiology research support and writing the paper; Herbert Kiss: Corresponding author, organisation of the research and writing the paper; Ljubomir Petricevic: Senior author, organisation of the research and writing the paper.

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